REMARKS

Specification Amendments:

The specification has been amended to insert references to the SEQ ID numbers in the sequence listing (pages 71-90 of the specification as filed). Therefore, no new matter has been added by these amendments. The Examiner is hereby requested to enter these amendments.

Conclusions:

Applicants request that the above amendments be entered prior to examination of this application. Early and favorable examination is hereby requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Ping F. Hwung
Registration No. 44,164

P.O. Box 1404
Alexandria, Virginia 22313-1404
(650) 622-2300

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Attachment to Preliminary Amendment dated March 11, 2003
Marked-up Copy of Amendments in the Specification

Please amend paragraph 1 on page 7 as follows:

FIG. 1 and 2 show the native DNA sequences encoding, and the deduced amino acid sequences of, human basic FGF (SEQ ID NO: 1) and acidic FGF (SEQ ID NO: 3), respectively.

Please amend paragraph 2 on page 7 as follows:

FIG. 3 shows a comparison of the amino acid sequences for human acidic (SEQ ID NO: 4) and basic (SEQ ID NO: 2) FGF and the various regions targeted for alteration, including potential heparin-binding domains and receptor-binding regions.

Please amend paragraph 3 on page 7 as follows:

FIG. 4 shows the construction of a synthetic tryptophan operon promoter and operator regulatory sequence (SEQ ID NOS: 5-6), and a restriction site map of plasmid pTRP-233.

Please amend the last paragraph on page 39, which continues on page 40, as follows:

There are only two amino acid differences between basic bovine FGF and human FGF, at position 123, where the bovine protein has Ser and the human protein has Thr, and at position 137, where the bovine protein has Pro and the human has Ser. These differences are the result of a single nucleotide difference in each case; therefore bovine CDNA may conveniently be modified by site directed mutagenesis as described below to encode the human protein, and, indeed, standard site-specific mutagenesis techniques were used to alter these codons. The lambda BB2 clone (ATCC No. 40196) was digested with EcoRI and the

1.4 kb region spanning the bFGF protein-encoding portion was ligated into the EcoRI site of M13mp8 and phage carrying the insert in the correct orientation were recovered. The in vitro mutagenesis was carried out in the presence of three oligonucleotides: the "universal" primer, a 17-mer; the mutagenic 16-mer 5'-GAAATACACCAGTTGG-3' (SEQ ID NO: 7); which alters the coding sequence at codon 123, and the mutagenic 17-mer 5'-ACTTGGATCCAAAACAG-3 (SEQ ID NO: 8), which alters the sequence at codon 137. The mutagenized phage was also subjected to a second round of in vitro primer-directed mutagenesis to create a HindIII site 34 bp downstream from the translation termination codon using the mutagenic 25-mer, 5'-TTTTACATGAAGCTTTATATTTCAG-3' (SEQ ID NO: 9). The resultant mutated DNA was sequenced by dideoxynucleotide sequence analysis (Sanger et al, supra) to confirm that the desired mutagenesis had occurred, and the approximately 630 bp fragment spanning the FGF coding region was excised with HindIII and ligated into HindIII digested pUC13 to obtain the intermediate plasmid pJJ15-1.

Please amend the table on the bottom of page 40 (extending onto page 41) as follows:

Number	Sequence	
1670	5'-pAGCTTCATATGGCTGCTGGTTCTATCACTACC	(SEQ ID NO:10)
1623R	5'-pCTGCCAGCTCTGCCAGAAGACGGTGGTT	(SEQ ID NO:11)
1624R	5'-pCTGGTGCCTTCCCACCAGGTCACTTCAA	(SEQ ID NO:12)
1625R	5'-pAGACCCAAAACGTCTGTACTGCAAAAAC	(SEQ ID NO:13)
1680	5'-pGGTGGTTTCTTCCTGCGCA	(SEQ ID NO:14)
1679	5'-pTAGAACCAGCAGCCATATGA	(SEQ ID NO:15)
1622	5'-pTCTTCTGGCAGAGCTGGCAGGGTGA	(SEQ ID NO:16)
1619	5'-pACCTGGTGGGAAGGCACCAGAACCACCG	(SEQ ID NO:17)
1626	5'-pAGTACAGACGTTTTGGGTCTTTGAAGTG	(SEQ ID NO:18)
1673	5'-pAGCTTGCGCAGGAAGAAACCACCGTTTTTGC	(SEQ ID NO: 19)

Please amend the paragraph starting at page 41, line 5, as follows:

Construction of Synthetic Gene for the Amino Terminal Region of bFGF (SEQ ID NOS: 20-21):

Please amend the first complete paragraph on page 46 as follows:

In this example, cysteine residues at positions 34 and 101 of the human basic FGF protein were changed to serine residues thereby producing a double mutation. Approximately 2 micrograms each of the mutagenic 23-mer 5'-ACGTCTGTACTCCAAAAACGGTG-3' (SEQ ID NO: 22) (#2222); which alters the sequence at codon 34, and the mutagenic 23-mer 5'-TACAGACGAGTCTTTCTTTTTTG-3' (SEQ ID NO: 23) (#2323); which alters the sequence at codon 101 were incubated in 50 ul of 1.times.kinase/ligase buffer (7 mm Tris-HCl pH 7.6, 10 mm MgCl.sub.2, 5 mm dithiothreitol) with 1 mM ATP and 5 units T.sub.4 polynucleotide kinase for 30 minutes at 37.degree. C. The phosphorylated oligonucleotides were diluted two-fold into 1 mM Tris-HCl, pH 8.0 and 1 mM EDTA.

Please amend Table 1 on page 48 as follows:

TABLE 1

bFGF Analog[+]	Oligonucleotide[#]	Number*
1) bFGF-C78S	5'-pCAAAGGAGTGTCTGCAAACCGTT (SEQ ID NO:24)	2217
2) bFGF-C96S	5'-pAGCTTCTAAATCTGTTACAGACG (SEQ ID NO:25)	2218
3) bFGF-C78/96S		2218/2217
4) bFGF-C34/78/96/101S		2217/2218/2222/2323
25	5'-pACGTCTGTACTCCAAAAACGGTG (SEQ ID NO:26)	2222
	5'-pTACAGACGAGTCTTTCTTTTTTG (SEQ ID NO:27)	2323
5) bFGF-C34/78/96S		2222/2218/2217
6) bFGF-C78/96/101S		2217/2218/2323
7) bFGF-C34/78/101S		2222/2217/2323

8) bFGF-C34/78S	2222/2217
9) bFGF-C34/101S	2222/2323

Please amend the table in Example 9 (pages 54 and 55) as follows:

Analog	Oligonucleotide	Number
bFGF-K35S	5'-pGTCTGTACTGCTCAAACGGTGGTT (SEQ ID NO:28)	2553
bFGF-R42L	5'-pTTTCTTCCTGCTCATCCACCCCG (SEQ ID NO:29)	2327
bFGF-D46A	5'-pCATCCACCCCGCCGGCCGAGTGG (SEQ ID NO:30)	2221
bFGF-R48L	5'-pCCCCGACGGCCTAGTGGACGGGG (SEQ ID NO:31)	2454
bFGF-R48A	5'-pACCCCGACGGCGCAGTGGACGGGG (SEQ ID NO:32)	2555
bFGF-D50A	5'-pCGGCCGGAGTGGCCGGGGTCCGCG (SEQ ID NO:33)	2224
bFGF-V52K	5'-pGAGTGGACGGGAAACGCGAGAAGAG (SEQ ID NO:34)	2491
bFGF-R53L	5'-pGGACGGGGTCCTCGAGAAGAGCG (SEQ ID NO:35)	2220
bFGF-K55M	5'-pGGTCCGCGAGATGAGCGACCCAC (SEQ ID NO:36)	2223
bFGF-K55I	5'-pGGTCCGCGAGATAAGCGACCCACA (SEQ ID NO:37)	2567
bFGF-D57A	5'-pCGAGAAGAGCGCCCCACACATCA (SEQ ID NO:38)	2225
bFGF-H59N	5'-pGAGCGACCCAAACATCAAACTAC (SEQ ID NO:39)	2383
bFGF-R9OT	5'-pAGAAGATGGAACTTTACTAGCTTC (SEQ ID NO:40)	3088
bFGF-D99A	5'-pATGTGTTACAGCAGAGTGTTTCT (SEQ ID NO:41)	2381
bFGF-E100A	5'-pGTTACAGACGCCTGTTTCTTTTTTG (SEQ ID NO:42)	2549
bFGF-E100S	5'-pGTGTTACAGACAGTTGTTTCTTTTT (SEQ ID NO:43)	2380
bFGF-E105S	5'-pGTTTCTTTTTTTCACGATTGGAGT (SEQ ID NO:44)	2556
bFGF-R106L	5'-pCTTTTTTGAACTATTGGAGTCTA (SEQ ID NO:45)	2494
bFGF-E108A	5'-pTGAACGATTGGCATCTAATAACTA (SEQ ID NO:46)	2554
bFGF-Y112A	5'-pAGTCTAATAACGCAAATACTTACCG (SEQ ID NO:47)	2450
bFGF-N113S	5'-pCTAATAACTACAGTACTTACCGG (SEQ ID NO:48)	2452
bFGF-R116T	5'-pCAATACTTACACTTCAAGGAAATA (SEQ ID NO:49)	3091
bFGF-R118L	5'-pCAATACTTACCTGTCAAGGAAAT (SEQ ID NO:50)	2483
bFGF-K119S	5'-pACCGGTCAAGGTCTTACACCAGTTG (SEQ ID NO:51)	2548
bFGF-(41-43)	5'-pGGTGGTTTCTTCCACCCCGACGGC (SEQ ID NO:52)	2336
bFGF-(49-51)	5'-pCCCGACGGCCGAGTCCGCGAGAAG (SEQ ID NO:53)	2335

bFGF-(62-64)	5'-pCCACACATCAAACAAGCAGAAGAG (SEQ ID NO:54)	2334
bFGF-(83-85)	5'-pGCAAACCGTTACAAAGAAGATGGA (SEQ ID NO:55)	2333
bFGF-(105-107)	5'-pTGTTTCTTTTTTGAGTCTAATAAC (SEQ ID NO:56)	2332
bFGF-(112-114)	5'-pGAGTCTAATAACTACCGGTCAAGG (SEQ ID NO:57)	2337

Please amend the table in Example 10 as follows:

Analog	Oligonucleotide	Number
bFGF-K27M	5'-pAGGTCACTTCATGGACCCAAAACG (SEQ ID NO:58)	2487
bFGF-K30A	5'-pTCAAAGACCCAGCACGTCTGTACT (SEQ ID NO:59)	2566
bFGF-R31S	5'-pAAGACCCAAAATCTCTGTACTGCA (SEQ ID NO:60)	2568
bFGF-D28K	5'-pGTCACTTCAAAAAGCCAAAACGTCT (SEQ ID NO:61)	2480
bFGF-R118L	5'-pCAATACTTACCTGTCAAGGAAAT (SEQ ID NO:62)	2483
bFGF-K35S	5'-pGTCTGTACTGCTCAAACGGTGGTT (SEQ ID NO:63)	2553
bFGF-K128S	5'-pATGTGGCACTGGAGCGAACTGGGCA (SEQ ID NO:64)	2545
bFGF-K128E	5'-pATGTGGCACTGGAGCGAACTGGCCA (SEQ ID NO:65)	3332
bFGF-R129T	5'-pGGCACTGAAAACTACTGGGCAGT (SEQ ID NO:66)	3087
bFGF-k128E/R129T		
bFGF-K134S	5'-pCTGGGCAGTATTCTCTTGGATCCAA (SEQ ID NO:67)	3212
bFGF-K138S	5'-pAACTTGGATCCTCTACAGGACCTGG (SEQ ID NO:68)	3215

Please amend paragraph 1 on page 62 as follows:

The blunted NdeI-HindIII FGF fragment from pUC9delH3-PTSF-3 was subcloned into the SmaI-HindIII site of M13mp18. An oligo was used to introduce a new NdeI site in the FGF molecule at amino acid 25 using in vitro mutagenesis. The new NdeI site serves as both a new restriction site for subcloning the FGF fragment and also as a new translational start site for the shortened form of FGF. The mutagenic oligo used has the sequence:

5'-TTG GGT CTT TGA AGT GCA TAT GTG GGA AGG CAC CAG (SEQ ID NO: 69)